Copeptin as a Marker and Vitamin D as a Protector to Some Metabolic and Hormonal Changes in Both Lean and Obese Polycystic Ovary Syndrome Rat Model

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common causes of female infertility. It is commonly linked to insulin resistance (IR), obesity, hyperlipidaemia, and type II diabetes. Copeptin has been linked to insulin resistance and the development of atherosclerosis. There have been some debates about copeptin levels and their relationship to hormonal and metabolic changes in PCOS. Vitamin D deficiency is common in PCOS and may contribute to the disease's pathophysiology.

Aim of the work: The purpose of this study was to estimate serum copeptin levels in letrozole-induced PCOS in both lean and obese female rats, as well as to investigate the relationship between serum copeptin levels and some metabolic and hormonal parameters in PCOS, as well as to determine the effect of vitamin D administration on different groups and its relationship to PCOS with copeptin serum levels.

Material and Methods: This study included 64 young virgin female albino rats of the local strain. They were divided into eight equal groups: lean control, lean PCO, lean vitamin D supplemented, lean PCO supplemented with vitamin D, obese control, obese PCO, obese vitamin D supplemented, and obese PCO supplemented with vitamin D. For 7 weeks, lean groups were fed standard laboratory chow, while obese groups were fed a high-fat chow. PCO groups were induced by orally supplementing with letrozole (Daily 0.5 mg/kg dissolved in water by oral gavage) for 21 days, and vitamin D groups were supplemented orally for 70 days.

Fasting serum copeptin, luteinizing hormone (LH), follicular stimulating hormone (FSH), testosterone, estradiol, progesterone, insulin, glucose, triglycerides, cholesterol, low density lipoprotein LDL, and high-density lipoprotein HDL levels were measured. In addition, at the end of the experiment, all groups had their BMI and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) calculated.

Results: When compared to both lean and obese control groups, serum copeptin levels were significantly higher, while vitamin D levels were significantly lower in both lean and obese PCOS groups. Furthermore, when compared to the lean PCOS group, the obese PCOS group had significantly higher levels of copeptin and lower levels of vitamin D. Furthermore, there was a significant positive correlation between serum copeptin and BMI, insulin, glucose, HOMA IR, cholesterol, TG, LDL, VLDL, LH, and testosterone. It had a significant inverse relationship with

Vitamin D, HDL, Progesterone, and estrogen. Supplementing with vitamin D improved insulin sensitivity, dyslipidaemia, and some hormonal changes in PCOS.
Conclusion: Copeptin is associated with many metabolic and hormonal changes accompanying polycystic ovary syndrome and may have a role in the development of this disorder and associated comorbidities. In addition, Vitamin D is negatively correlated with copeptin and its deficiency is linked to PCO, so vitamin D maybe a suitable supplement to prevent the associated cardiometabolic disorders in PCOS in both lean and obese.
Key words: copeptin -PCOS- lean – obese-Vitamin D

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Introduction:

A diverse endocrine disorder called polycystic ovarian syndrome (PCOS) affects women of reproductive age and is characterized by abnormalities in androgen secretion. This may cause cyclicity to be disrupted and the formation of polycystic ovaries [(1).] These can impede follicular growth, cause oligo/anovulation, and result in infertility. The Rotterdam criteria are used to determine whether a person has PCOS, and they require the presence of two of the three symptoms of ovulatory dysfunction, polycystic ovarian morphology, and clinical or biochemical hyperandrogenism [(2).] Since direct measurement of arginine vasopressin (AVP) can be technically challenging, Copeptin measurements have allowed for large-scale epidemiologic studies linking activation of the vasopressin system to stress. Copeptin, the C-terminal provasopressin fragment, directly mirrors vasopressin level and reflects the individual stress level because of its hemodynamic osmoregulatory effects and is more stable in plasma and serum [(3)] Vitamin D deficiency is described in women with PCOS, and some studies suggested that the vitamin D pathway may play a role in the onset of PCOS and its symptoms, which include hirsutism, ovulatory dysfunction, IR, and cardiovascular diseases [(4).] Recently, copeptin appears to play a significant role in metabolic response and the subsequent development of PCOS [(5).] Vitamin D exerts its effects in various organs via interacting with the nuclear vitamin D receptor, including immune cells, parathyroid glands, the pancreas and placenta, uterus, ovaries, and testes [(6).] Only a few studies have examined the connection between vitamin D levels and blood copeptin levels, and this work may be the first to do so using a rat model of PCOS. As a result, this study attempts to clarify this relationship by measuring serum copeptin levels in lean and obese female rats given letrozole to induce PCOS. It also investigates the relationship between serum copeptin levels and various metabolic and hormonal parameters in PCOS, as well as the effects of vitamin D supplementation on different groups and how it relates to serum copeptin levels.

MATERIAL AND METHODS:

This study included 64 young healthy virgin female albino rats of the local strain, aged 6 weeks and weighing 150-200 gm. They were obtained from Zagazig University's animal house faculty of veterinary medicine. The rats were housed in steel wire cages (8/cage) in the animal house of

Zagazig University's faculty of medicine under sanitary conditions. The rats had free access to water and food, were kept at room temperature, and followed a 12-hour light/dark cycle. The experimental protocol was approved by the physiology department and the local medical ethics committee at Zagazig University's faculty of medicine (Institutional Review Board, IRB).

The animals were divided into two main groups:

Group A: "lean group (n=32)": The animals in this group were fed a standard laboratory chow diet that contains (25.8% protein, 62.8% carbohydrates, and 11.4% fat (total 12.6 KJ/g) [7] and was further divided into four sub-groups: - (8 rats in each group)

• Group A1 (lean control): rats fed a regular normal laboratory chow diet and given 1ml of water by oral gavage for 21 days.

• Group A2 (lean PCO): rats fed a standard laboratory chow diet and orally supplemented with letrozole (0.5 mg/kg dissolved in water by oral gavage) for 21 days.

• Group A3 (lean + vitamin D): For 70 days, rats were fed a regular normal laboratory chow diet and supplemented with vitamin D orally (V drop) at a rate of 1000IU/Kg/day.

. Group A4 (lean PCO+ vitamin D): rats fed a regular normal laboratory chow diet supplemented with letrozole (Daily 0.5 mg/kg dissolved in water by oral gavage) for 21 days and vitamin D (V drop) 1000IU/Kg/day for 70 days.

Group B (n=32 rats): "A high fat diet induced obesity group": For 7 weeks, rats in this group were fed a high-fat chow diet that included (16.45% protein, 25.6% carbohydrate, and 58.0% fat (total 23.4 KJ /g) in the form of cotton seed oil added to the laboratory chow diet [8] and was further subdivided into four sub-groups: (8 rats in each group)

• Group B1 (obese control): rats fed a high fat diet for 7 weeks and then given 1ml of water by oral gavage for 21 days.

• Group B2 (Obese PCO): rats were fed a high fat diet for 7 weeks and then given letrozole (0.5 mg/kg dissolved in water by oral gavage) for 21 days.

• Group B3 (obese + vitamin D): rats were fed a high fat diet for 7 weeks and given 1000IU/Kg/day vitamin D orally (V drop) for 70 days.

• Group B4 (obese PCO+ vitamin D): rats fed a high fat diet for 7 weeks were given letrozole (0.5 mg/kg dissolved in water by oral gavage) for 21 days and given vitamin D orally (V drop) 1000IU/Kg/day for 70 days.

Sexual cycle determination

During the treatment period, smears were obtained daily by vaginal washing with saline and fresh unstained samples were evaluated microscopically; cycles lasting 4 to 5 days were considered regular (9).The four phases of the estrus cycle according to Marcondes et al [10] and Goldman et al [11] as follow:

- The vaginal smear shows many live epithelia with smooth margins during the proestrus phase.

The vaginal smear shows large cornified (keratinized) cells with irregular margins during the estrus phase.
-The vaginal smear reveals many cornified cells as well as leukocyte infiltration during the met estrus phase.
-During the diestrus phase, the vaginal smear reveals the absence of cornified cells and the presence of small leukocytes.
In this procedure, persistent estrus was defined as the presence of cornified cells in the smears for at least two consecutive 4-day estrus cycles, indicating the development of follicular cysts. All rats have regular cycles at the start of the experiments [12].
Body mass index (BMI) was calculated for all groups at the start and end of the study period using the equation: body weight (gm)/length2 (cm2) (nose to anus length) [13].
Letrozole was obtained from (Novartis Pharma S.A.E. Amiria, Cairo) and was administered orally every day at 0.5 mg/kg dissolved in water for 21 days [14].
For 70 days, the vitamin D-treated groups received cholecalciferol (V drop oral drops, Novartis Co, Switzerland) via gavage in doses of 1000IU/Kg/day [15].

Blood testing:
At the end of the experiment, all rats were sedated with diethyl ether. The rats were fasting with free access to water while the former samples were collected from the orbital sinus (control samples taken during the estrus phase) and centrifuged at 3000 X for 15 minutes [16]. The serum was separated and stored at -70 ° C. Repeated freezing and thawing were avoided [17] until the following parameters were determined: Tietz et al [18] estimated serum LH, FSH, estradiol, progesterone, and testosterone levels using rat kits: BC-1031, BC-1029, BC-1111, BC-1113, and BC-1115, respectively, BioCheckInc 323 Vintage Park Dr. Foster City, CA - 94404,
Serum glucose and insulin levels can be measured using enzyme-linked immunosorbent assays (ELISA), according to Bonora et al [19]. (Spinreact Ltd, Girona, Spain- Ref. ID: MD41011, Diametra Ltd, Garibaldi, Italy- Ref. DKO076).
According to Sun et al [20], the homeostasis model assessment (HOMA-IR) index was calculated as follows: [HOMA-IR] = fasting serum glucose (mg/dl)/405, fasting serum insulin (IU/ml)/405, fasting serum insulin (IU/ml)/405, The following is an estimate of the lipid profile: Tietz et al. define total serum cholesterol as [21] [Serum TG levels were calculated by Fossati et al [22], serum HDL levels by Nauck et al [23], and serum LDL levels by Friedewald et al [24] as follows: LDL = TC minus HDL minus TG/5 (Biosource Europe provided kits for estimating serum, cholesterol, TG, and HDL levels.)S.A.Belgium).

serum copeptin levels determined as follows: The kit employs a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to determine the level of Rat Copeptin in samples (USCN) [25]

Serum vitamin D levels are determined as follows:

Serum 25-hydroxy vitamin (25(OH)D) concentration, which is the main circulating form of VD, was used to determine VD status. The 25(OH) VD concentration was determined using a commercial ELISA kit (Eagle Biosciences, Immundiagnostik, and MicroVue) in conjunction with a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method for measuring serum 25(OH)D concentration [26]

Histopathological examinations

The ovaries were dissected and fixed in 10% buffered formalin for 6 hours at room temperature before being washed in a phosphate buffered saline solution, dehydrated in an ascending series of ethanol, cleared in xyline, and embedded in paraffin. For light microscopy, 5m thick sections were mounted in slides previously treated with 3-aminopyropyl triethoxysilane and stained with hematoxylin and eoisin [27]

Statistical investigation

The data was presented as mean SDM. The one-way ANOVA test was used to determine statistical significance. P values of 0.05 or less were considered significant. SPSS version 27 for Windows (SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

The data was presented as mean SDM. The one-way ANOVA test was used to determine statistical significance. P values of 0.05 or less were considered significant. SPSS version 27 for Windows (SPSS Inc. Chicago, IL, USA) was used for statistical analysis.

Pearson's correlation analysis was used to demonstrate the relationships between serum copeptin and the studied metabolic parameters among different groups. Pearson's correlation was considered significant when P values were less than 0.05.

RESULTS

Histopathological findings Ovaries from the lean groups (A1, A2, A3 and A4 groups) histologically (under 40_ magnification) group A1&A3 showed ovarian tissues with normal structure with mature follicular cysts and corpus luteum, group A2 showed ovarian tissues with follicles lined by thick granulosa cells and multiple large follicular cysts lined by flat epithelium. While group A4 showed the ovarian tissues with better follicular maturation than group (A2) by presence of grafian follicles and stages up to corpus luteum and decreased number of cysts with flattened epithelium. In ovaries from obese groups (B1, B2, B3 and B4 groups) histologically (under 40 magnification), group B1 showed ovarian tissues with some follicular cysts with thin and flattened granulosa cell lining, group B2 showed ovarian tissue with evident cystic structures with many large cystic follicles lined by flat epithelium. While group B3 and B4 showed ovarian tissue with decreased follicular cysts than group (B1&B2) with different maturation stages including grafian follicles and corpus luteum. **(Figure 1&5).**

This study revealed that in lean PCOS group (A2) there were significant high levels of serum copeptin, Insulin, Glucose, HOMA IR, cholesterol, TG, LDL, VLDL LH, testosterone and BMI when compared with that of lean control group (A1) (P value: < 0.001, <0.001, <0.001, < 0.001<0.001, <0.001, <0.001, <0.001, <0.001, <0.001< 0.001 respectively). While there were significant low levels of HDL, serum estrogen and progesterone, vitamin D in group (A2) compared to control group (A1) (P value: < 0.001, < 0.001, <0.001, <0.001, respectively) and no change in FSH level between group (A2) and (A1).

Also, this study showed in lean PCO + vitamin d group (A4) there was significant decrease in serum copeptin, glucose, insulin, HOMA IR, cholesterol, TG,VLDL, LH, testosterone and BMI (P value: < 0.01, < 0.001, <0.001, <0.04, < 0.001 < 0.001, <0.001< 0.001, <0.001, <0.02, respectively) and significant increase in levels of HDL, progesterone, and estrogen (P value: < 0.04, <0.02, < 0.001 respectively) when compared to lean PCO group (A2).

Moreover, in obese PCOS group (B2) there were significant high levels of serum copeptin, insulin, Glucose, HOMA IR, cholesterol, TG, LDL, VLDL, LH, testosterone and BMI when compared with that of obese control group(B1), (Pvalue:<0.001, <0.001, <0.001 ,<0.001<0.001 <0.001<0.001,<0 .001<0.001<0.001<0.001 respectively). While there were significant low levels of HDL, serum estrogen and progesterone, vitamin D in group (B2) compared to obese control group (B1) (P value: < 0.001, < 0.001, <0.001< 0.001, respectively) and no change in FSH level between group (B2) and (B1).

while this study revealed that in obese PCO + vitamin d group (B4) there was significant decrease in serum insulin, HOMA IR and LH levels (P value: <0.002, <0.001, <0.001, respectively) and no significant change in serum copeptin, BMI, glucose, HDL, FSH, progesterone, estrogen, and testosterone when compared to obese PCO group (B2)

copeptin showed significant positive correlation among lean groups **(Figure 2&3)** with BMI, insulin, glucose, HOMA IR, cholesterol, TG, LDL, VLDL, LH and testosterone (r= 0.62** p <0.001**, r= 0.92** p <0.001**, r= 0.71** p <0.001**, r= 0.86** p <0.001**, r= 0.79** p <0.001**, r= 0.90** p <0.001**, r= 0.53** p <0.002*, r= 0.87** p <0.001**, r= 0.87** p <0.001**, r= 0.68** p <0.001**, respectively) while Copeptin showed significant negative correlation with HDL, Vit D, Progesterone, and Estrogen r= -0.76** p <0.001**, r= -0.80** p <0.001**, r= -0.78**, p <0.001** r= -0.87** p <0.001** respectively) in lean groups. **(Figure 4)**

Moreover, copeptin showed significant positive correlation among obese groups **(Figure 6&7)** with BMI, insulin, glucose, HOMA IR, cholesterol, TG, LDL, VLDL, LH and testosterone (r= 0.31** p <0.04*, r= 0.58** p <0.001**, r= 0.59** p <0.001**, r= 0.56** p <0.001*, r= 0.75** p <0.001**, r= 0.76** p <0.001**, r= 0.50** p <0.003*, r= 0.75** p <0.001**, r= 0.72** p <0.001**, r= 0.49** p <0.005*, respectively) while Copeptin showed significant negative correlation with HDL, Vit D, Progesterone, and Estrogen r= -0.71** p <0.001**, r= -0.64** p <0.001**, r= -0.70** p <0.001**, r= -0.69** p <0.001** respectively) in obese groups **(Figure 8)**

Table (1): All studied parameters among the lean groups:

Variable	Lean control	Lean PCO	Lean +Vitamin D	Lean PCO +Vitamin D
BMI (KG/m2)	0.49±0.03	0.57±0.04 ^{a**}	0.52±0.04 ^{b*}	0.54±0.04 ^{a*,b*,c*}
CPP (ng/l)	160.5±50.58	600±80.44 ^{a**}	205±35.18 ^{b**}	444.5±52.65 ^{a**,b**,c**}
Insulin (mIU/ml)	36.66±7.33	97.80±5.09 ^{a**}	42.98±7.59 ^{b**}	71.32±9.15 ^{a**,b**,c**}
Glucose (mg/dl)	116.23±3.90	136.06±4.34 ^{a**}	120.38±7.21 ^{b**}	129.35±8.71 ^{a**,b*,c*}
HOMAIR	17.9±2.46	31.63±3.06 ^{a**}	21.12±1.65 ^{b**}	25.78±2.97 ^{a**,b*,c*}
Cholesterol (mg/dl)	85.64±3.14	102.83±5.02 ^{a**}	88.24±3.26 ^{b**}	92.24±3.59 ^{a*,b*,c*}
Triglyceride (mg/dl)	105.49±4.95	158.89±9.24 ^{a**}	106.95±6.41 ^{b**}	126.7±7.59 ^{a**,b**,c*}
HDL (mg/dl)	61.01±4.65	48.12±3.33 ^{a**}	60.56±5.97 ^{b**}	52.34±4.0 ^{a**,b*,c**}
LDL (mg/dl)	86.07±6.83	95.30±5.89 ^{a**}	87.56±5.39 ^{b**}	91.66±6.44 ^{a*}
VLDL (mg/dl)	22.25±2.88	40.01±3.2 ^{a**}	21.89±2.35 ^{b**}	30.29±3.96 ^{a**,b**,c**}
Vit D (ng/ml)	34.3±4.63	20.08±2.97 ^{a**}	36.4±3.22 ^{b**}	28.34±4.61 ^{a*,b**,c**}
LH (IU/ml)	2.08±0.35	6.7±1.25 ^{a**}	2.03±0.43 ^{b**}	4.73±1.21 ^{a**,b**,c**}
FSH (IU/ml)	3.59±0.53	3.75±0.61	3.7±0.60	3.59±0.51
Progesterone (pg/ml)	8.09±0.71	5.09±1.07 ^{a**}	8.16±0.70 ^{b**}	6.19±1.04 ^{a**,b*,c**}
Estrogen (pg/ml)	32.22±3.78	15.34±2.91 ^{a**}	31.24±2.94 ^{b**}	24.82±4.14 ^{a**,b**,c**}
Testosterone (ng/ml)	2.58±0.53	5.5±1.76 ^{a**}	2.39±0.68 ^{b**}	3.9±1.11 ^{a*,b*,c*}

ANOVA test with LSD post hook, a: Significant versus Lean control, b: significant versus Lean PCO, C: significant versus Lean Vit D *: Significant (P<0.05) **: Highly significant (<0.001)

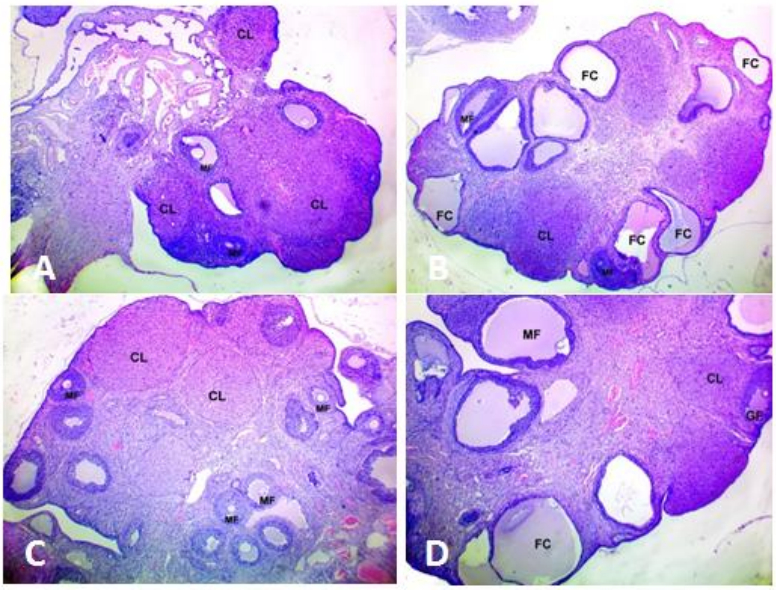

Figure (1): A: Group A1: section in the ovarian tissue with normal structure showing mature grafian follicle(MF) and corpus luteum (CL). **B: Group A2:** section in the ovarian tissue shows follicles lined by thick granulosa cells and multiple large follicular cysts(FC) lined by flat

epithelium. **C: Group A3**: section in the ovarian tissue showing normal different stages of maturation of ovarian follicles(MF) up to corpus luteum(CL). **D: Group A4:** section in the ovarian tissue shown few follicular cysts(FC) lined by flat epithelium, grafian follicles (MF) and stages up to corpus luteum(CL) **(H&E stain, x40 magnification)**

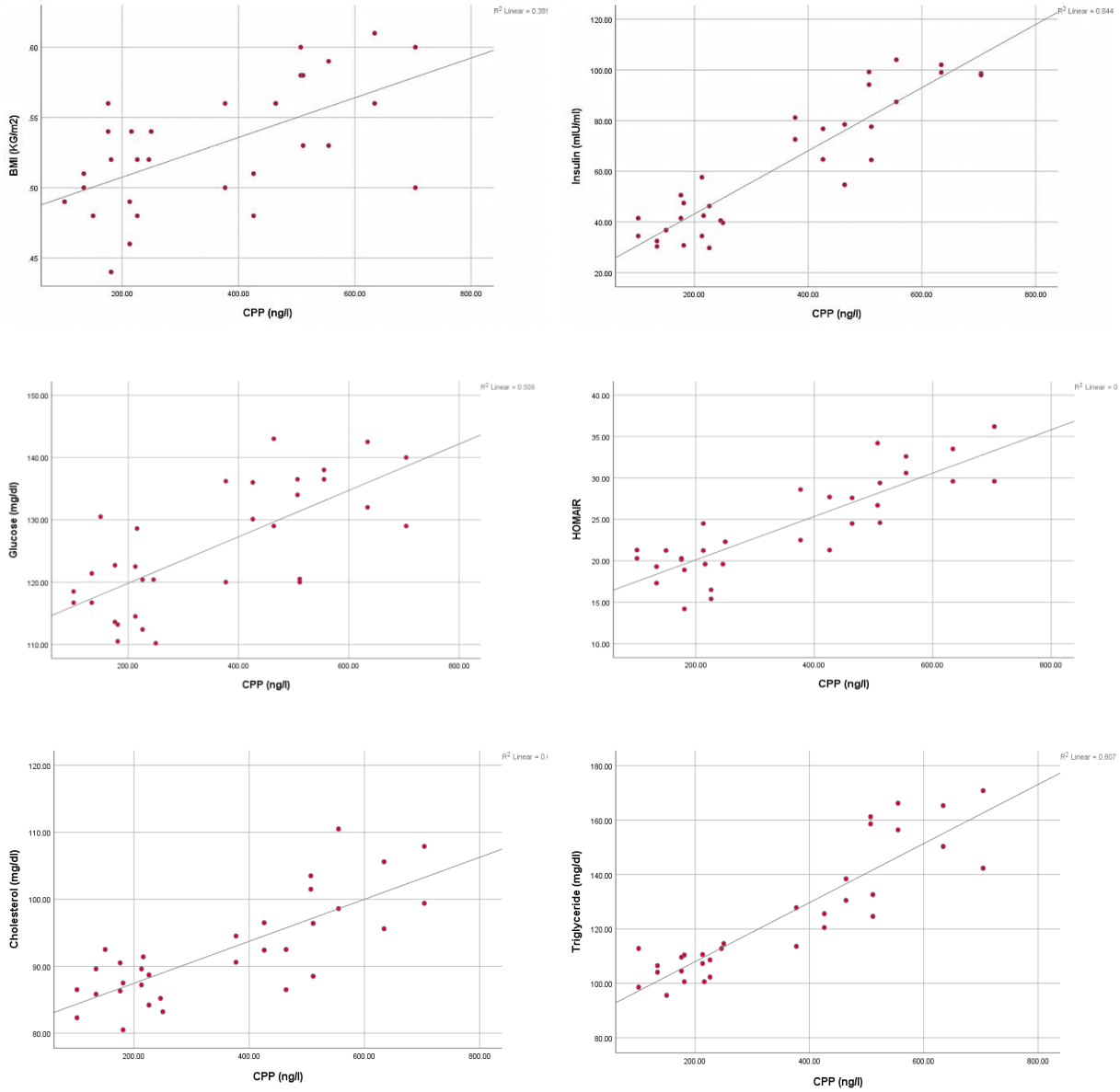

Figure 2: Correlation between Copeptin with BMI, insulin, glucose, HOMA IR, cholesterol, TG among lean groups

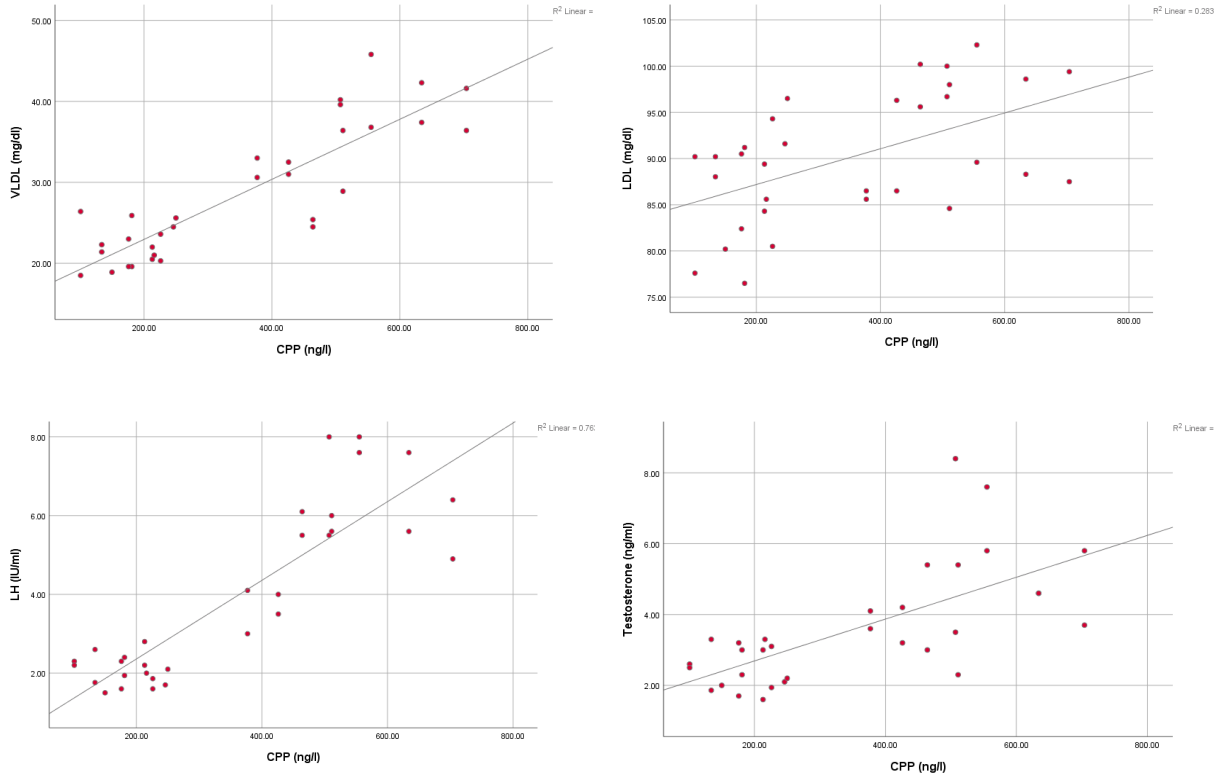

Figure 3: Correlation between Copeptin with LDL, VLDL, LH and testosterone among lean groups.

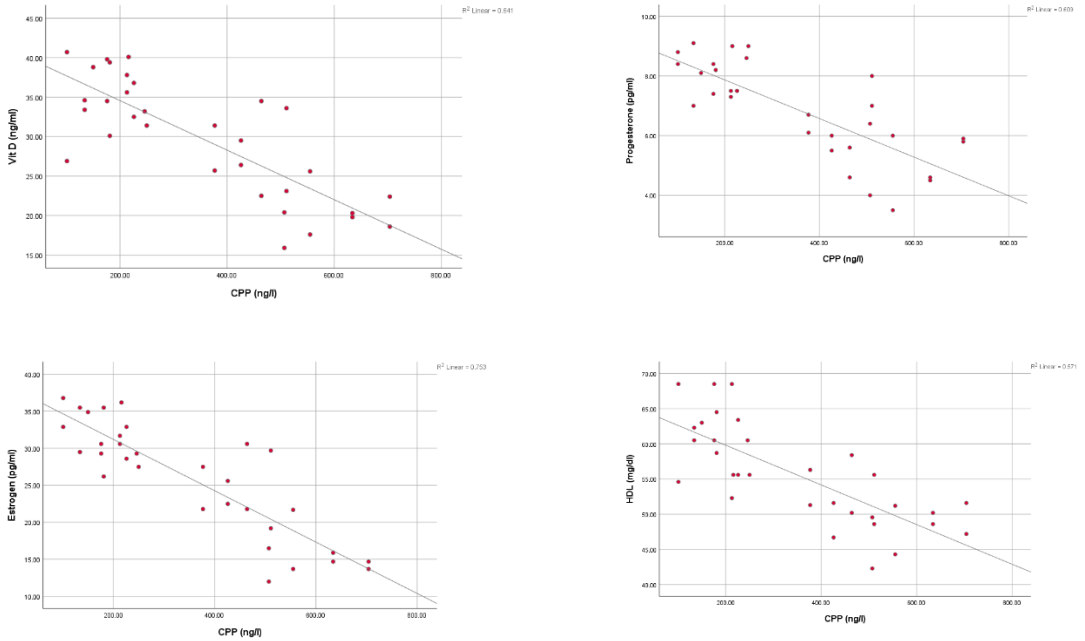

Figure 4: Correlation between Copeptin with HDL, Vit D, Progesterone, and Estrogen among lean groups.

Table (2): All studied parameters among obese groups:

Variable	Obese control	Obese PCO	Obese +Vitamin D	Obese PCO+ Vitamin D
BMI (KG/m2)	0.85±0.03	0.92±0.04 ^{a**}	0.87±0.04 ^{b*}	0.89±0.04 ^{a*,c*}
CPP (ng/l)	694.5±54.92	867.5±99 ^{a**}	691.75±54.21 ^{b**}	852.5±64.37 ^{a**,c**}
Insulin (mIU/ml)	117.88±8.90	183.51±9.53 ^{a**}	128.27±15.4 ^{b**}	143.89±12.24 ^{a**,b*,c**}
Glucose (mg/dl)	137.29±6.34	154.16±7.03 ^{a**}	140.68±3.34 ^{b**}	148.25±9.10 ^{a*,c*}
HOMAIR	43.01±3.48	57.56±4.36 ^{a**}	47.01±2.98 ^{b**}	50.84±3.85 ^{a**,b**,c*}
Cholesterol (mg/dl)	114.3±5.3	180.86±5.58 ^{a**}	126.01±3.28 ^{b**}	167.39±5.09 ^{a**,b**,c**}
Triglyceride (mg/dl)	200.25±10.19	297.23±20.06 ^{a**}	196.88±13.07 ^{b**}	262.65±11.09 ^{a**,b**,c*}
HDL (mg/dl)	44.61±3.8	29.05±4.27 ^{a**}	41.08±3.58 ^{b**}	31.05±3.85 ^{a**,c**}
LDL (mg/dl)	141.7±6.62	166.38±11.25 ^{a**}	144.75±5.95 ^{b**}	156.88±6.56 ^{a**,b*,c*}
VLDL (mg/dl)	41.33±2.03	76.66±4.88 ^{a**}	43.49±3.94 ^{b**}	68.64±3.29 ^{a**,b**,c**}
Vit D (ng/ml)	21.09±3.64	11.09±2.71 ^{a**}	20.79±19.6 ^{b**}	14.21±2.55 ^{a**,c**}
LH (IU/ml)	2.11±0.30	6.65±0.89 ^{a**}	2.01±0.39 ^{b**}	4.79±1.02 ^{a**,b**,c**}
FSH (IU/ml)	3.76±0.63	3.61±0.64	3.60±0.53	4.01±0.45
Progesterone (pg/ml)	7.85±0.72	4.44±1.25 ^{a**}	7.78±0.79 ^{b**}	5±1.10 ^{a**,c**}
Estrogen (pg/ml)	29.45±3.36	14.65±2.98 ^{a**}	29.55±2.19 ^{b**}	17.48±2.83 ^{a**,c**}
Testosterone (ng/ml)	2.59±0.85	5.44±1.82 ^{a**}	2.85±1.12 ^{b**}	4.68±1.32 ^{a*,c*}

ANOVA test with LSD post hook, a: Significant versus Obses control, b: significant versus Obese PCO, C: significant versus obese Vit D *: Significant (P<0.05) **: Highly significant (<0.001)

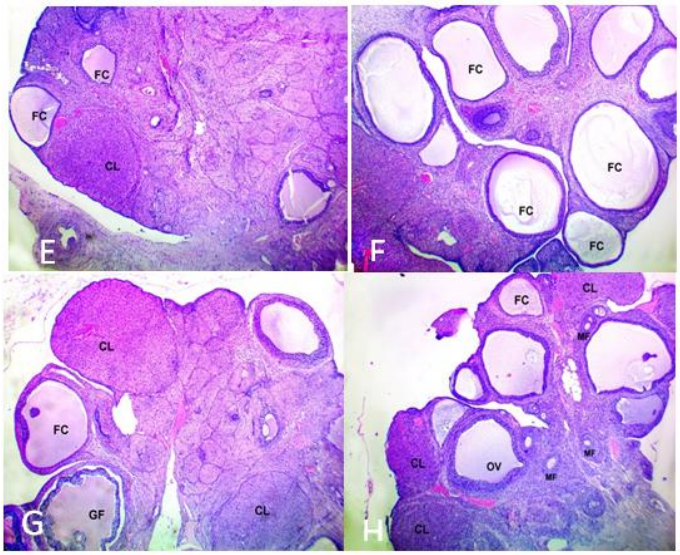

Figure (5). E: Group B1: section in the ovarian tissue shows some follicular cysts with thin and flattened granulosa cell lining and presence of corpus luteum(CL). **F:** Group B2: section in the ovarian tissue shows evident cystic structures with many large cystic follicles(FC) lined by flat epithelium. **G:** Group B3: section in the ovarian tissue shows follicular cysts(FC) with different

maturation stages including grafian follicles (MF) and corpus luteum(CL). **H:** Group B4: section in the ovarian tissue shows few follicular cysts and presence of corpus luteum(CL) **(H&E stain, x40 magnification).**

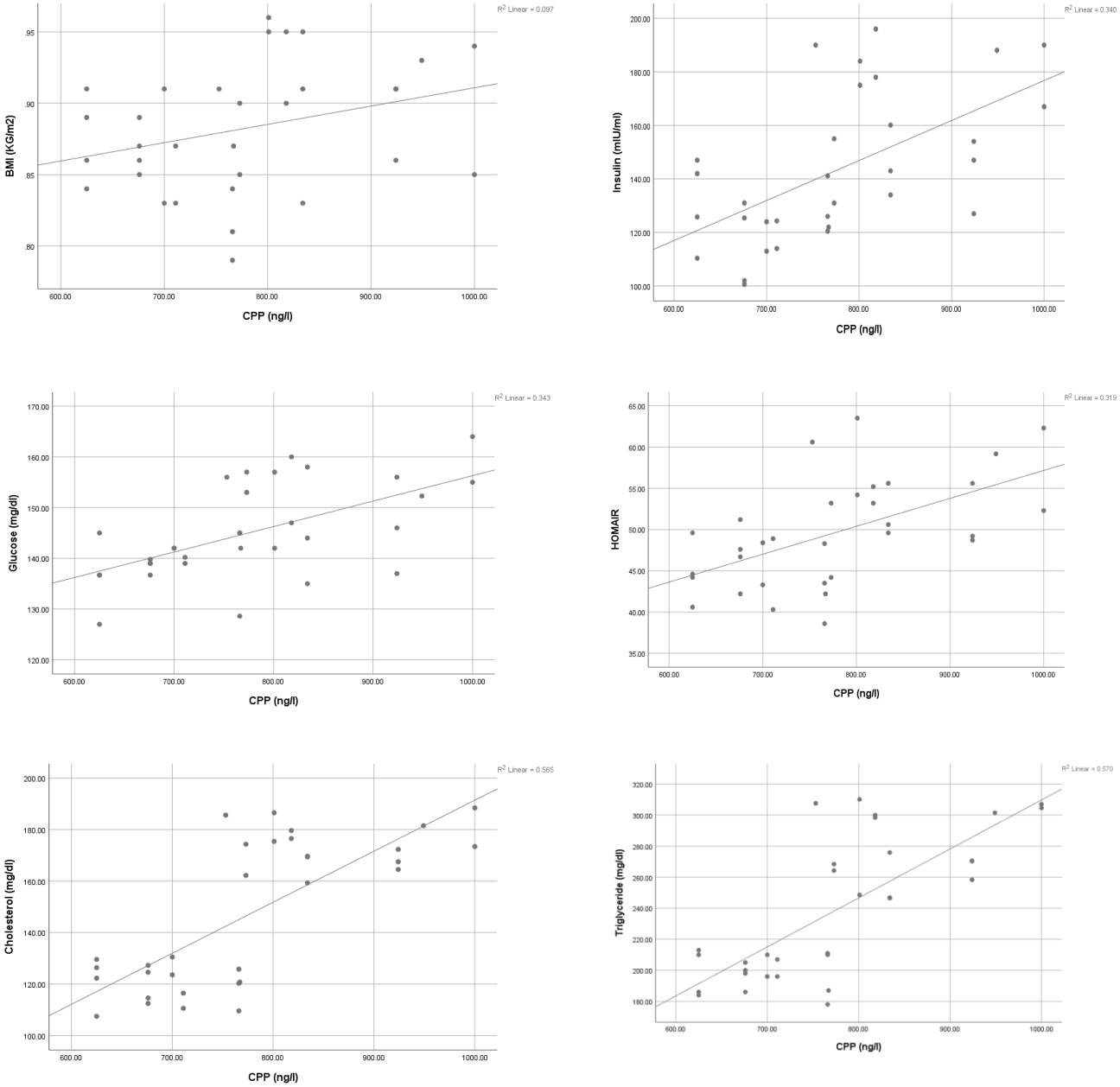

Figure 6: Correlation between Copeptin with BMI, insulin, glucose, HOMA IR, cholesterol, TG among obese groups

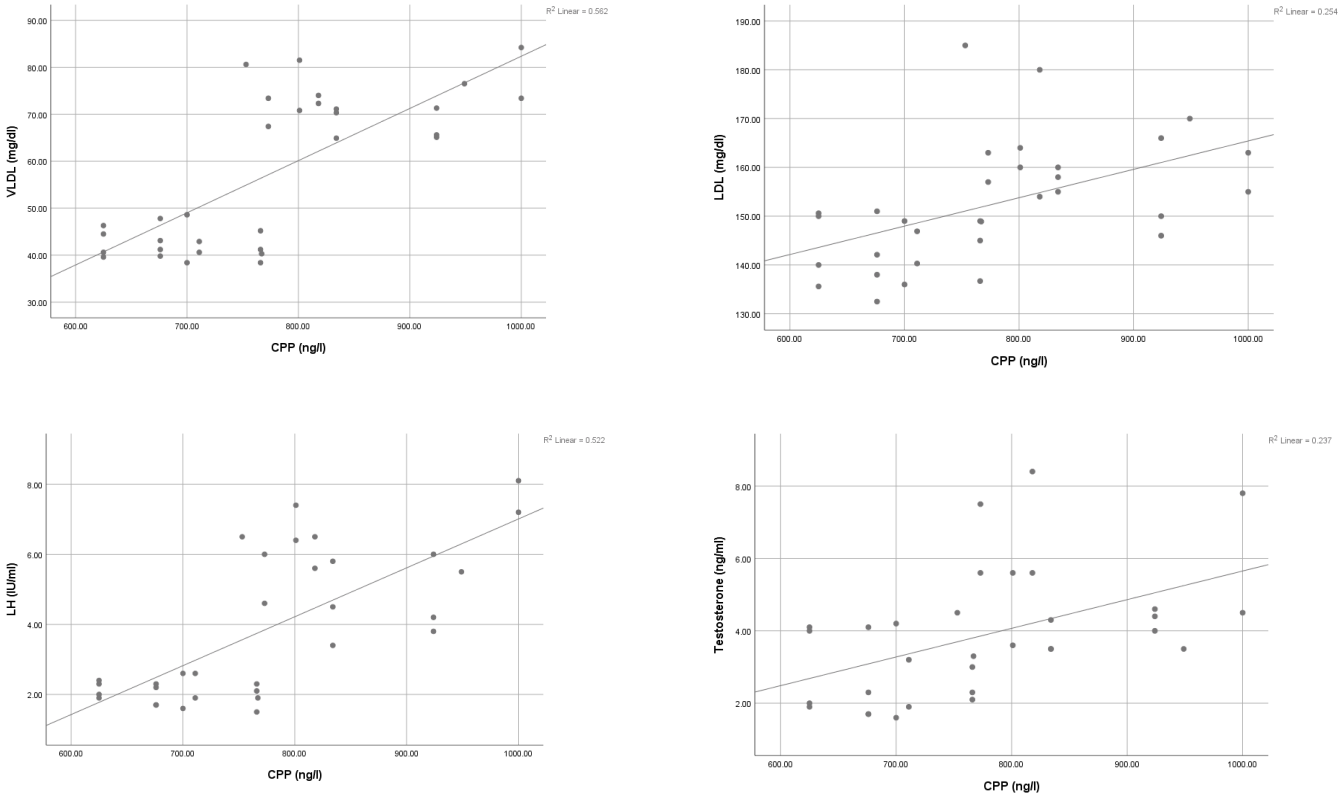

Figure 7: Correlation between Copeptin with LDL, VLDL, LH and testosterone among obese groups.

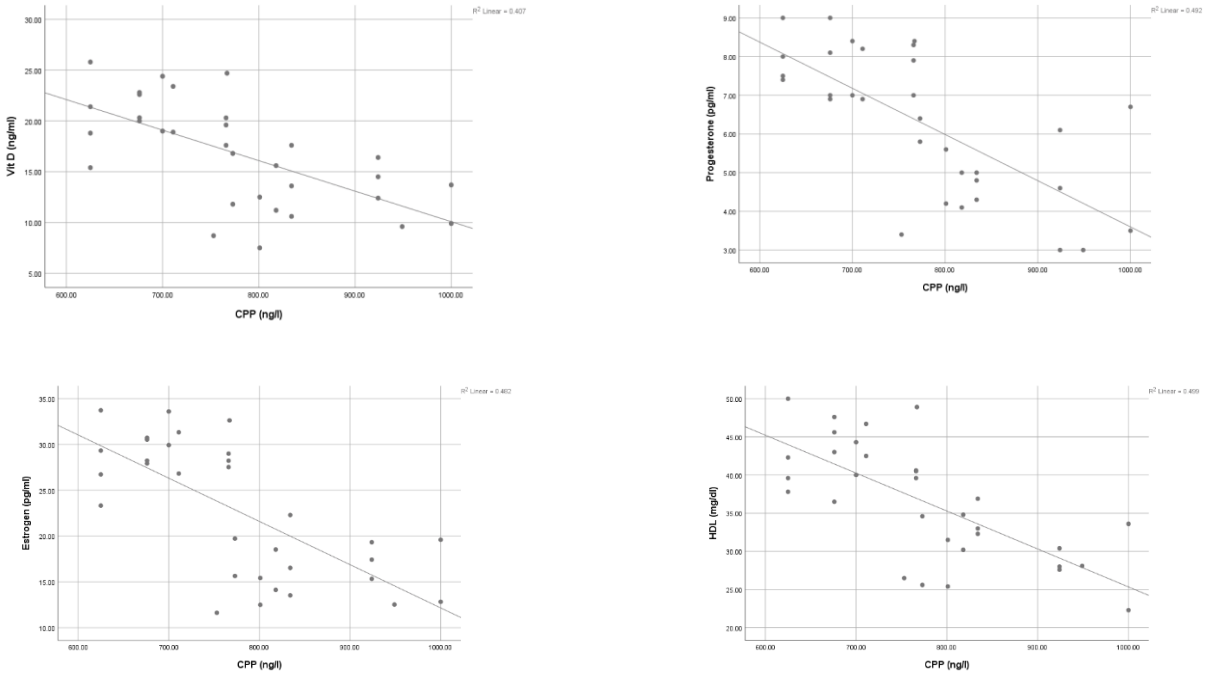

Figure 8: Correlation between Copeptin with HDL, Vit D, Progesterone, and Estrogen among obese groups.

DISCUSSION:

A diverse endocrine disorder called polycystic ovarian syndrome (PCOS) affects women of reproductive age and is characterized by abnormalities in androgen secretion. This may cause cyclicity to be disrupted and the formation of polycystic ovaries [28].

Women with PCOS have a higher cardiometabolic risk than the general population, which increases their chance of developing diabetes, hypertension, dyslipidemia, and cardiovascular diseases [29]. Additionally, the majority of PCOS patients are more likely to be obese and develop insulin resistance (IR) [30].

When there is persistent psychosocial stress, the posterior pituitary gland releases arginine vasopressin (AVP), also known as antidiuretic hormone, via stimulating the hypothalamic-pituitary-adrenal (HPA) axis and corticotropin-releasing hormone [31].

Due to its hemodynamic osmoregulatory effects and greater stability in plasma and serum than AVP, copeptin is currently recognized as a reliable and simple-to-measure substitute for AVP. Copeptin is the C-terminal provasopressin fragment that directly mirrors AVP level and reflects the individual stress level [32].

Numerous human tissues, including the ovary and endometrial, contain vitamin D receptors (VDRs), suggesting that these tissues play a role in female reproductive health. PCOS has been linked to abnormal calcium and phosphate metabolism as well as low vitamin D levels [33]

This study aimed to estimate serum copeptin levels in both lean and obese female rats that had letrozole-induced PCOS, as well as to investigate the relationship between serum copeptin levels and certain metabolic and hormonal parameters related to the condition. It also examined the effects of vitamin D administration on various groups and how they related to serum copeptin levels.

The signs of PCOS induced by letrozole in lean and obese rats were demonstrated by persistent estrus and histopathological features of cystogenesis, as well as significant hyperandrogenism (higher serum testosterone levels) and significant reductions in estradiol and progesterone levels in comparison to control groups [34]. Letrozole, which inhibits the enzyme cytochrome P450 aromatase that converts testosterone to estrogen, caused these symptoms [35].

With reference to the least significant difference (LSD) test used to compare the two groups, it was revealed that there was statistically significant rise in all obese groups vs all lean groups in the current study. Obese rats had BMI that were significantly higher than lean rats. When compared to lean controls, lean + vitamin D, and lean PCO + vitamin D, the lean PCO group exhibited a significant statistical rise, but the obese PCO group showed a significant statistical increase when compared to the obese controls, obese vitamin D, and obese PCO plus vitamin D.

This is in line with the findings of Widecka et al [36] who showed that the BMI of the control and PCO groups varied statistically.

Recent research on the relationship between vitamin D and obesity has revealed that vitamin D may potentially encourage the formation of fat tissue [37]. The generation of the active vitamin D metabolite is enhanced by PTH, which is raised in low vitamin D situations. However, PTH also

increases lipogenesis by increasing calcium input to adipocytes, which results in more fat and an increase in body weight [38]

Both the lean and the obese PCOS groups had significantly increased serum copeptin levels when compared to the control groups. Additionally, the level was significantly greater in the obese PCOS group compared to the lean PCOS group. When compared to the Lean PCO+Vitamin D group, the serum copeptin level in the Lean PCO group statistically increased.

Widecka et al [39] found that the plasma copeptin concentration was lowest in the control group and greatest in the PCOS, which is consistent with our results. According to Karbek et al [40], PCOS patients had mean copeptin levels that were greater than those of healthy control individuals. Additionally, Taskin et al [41] showed that the serum copeptin levels in the obese PCOS group were considerably greater than those in the nonobese PCOS and control groups. Additionally, Aly et al [42] showed that obese PCOS patients had considerably higher blood copeptin levels than control and non-obese groups.

High levels of serum copeptin may be attributed to PCOS, which carries a significant risk for the emergence of metabolic disturbances and cardiovascular abnormalities including metabolic syndrome [43]. This occurs frequently because insulin resistance is a key pathognomic characteristic of both metabolic syndrome and PCOS. In addition, several additional illnesses, such as high blood pressure, depression, insulin resistance, and high cholesterol, are linked to PCOS [44]

Copeptin appears to play a significant role in the metabolic response and the subsequent development of atherosclerosis in insulin-resistant, hyperandrogenemic PCOS patients since it is a neurohormone (NH) of the Arginine vasopressin AVP system [45], and Copeptin levels correlate to AVP levels in plasma [46] as a result [47].

In the current investigation, there was a strong positive association between serum copeptin levels, which were greater in fat than in lean rats, and BMI.

The correlations between copeptin and obesity may be caused by the possibility that AVP influences obesity by reducing brown adipose tissue activity or by having other effects on lipid metabolism [48]

A different study, however, suggested that while AP-36 and copeptin are not directly connected to the pathophysiology of PCOS, they may be linked as adipokines that are influenced by BMI [49].

These contradictory results could be explained by utilizing different blood samples (serum against plasma), various blood collection times (fasting state versus others), or different copeptin ELISA kits.

In this study, there was a highly significant difference between all lean groups and all obese groups in terms of insulin level, HOMA-IR, and glucose. Compared to all lean categories and obese groups, obese PCO is the greatest. In addition, Both the Lean PCO and obese PCO groups supplemented with vitamin D had significantly lower HOMA-IR, insulin and glucose level than other PCO groups.

Additionally, we notice that in all groups under study, there was a positive correlation between serum copeptin and the levels of glucose, insulin and HOMA-IR.
Widecka et al [50] found that the PCOS patients had the greatest plasma insulin levels and the highest plasma glucose concentrations, which is consistent with our results. Additionally, Behmanesh et al [51] showed that insulin, glucose, and IR serum concentrations in rats with PCOS were significantly higher than those in controls, and that these concentrations were significantly lower in the vitamin D-treated PCOS group than in the untreated PCOS group. This is understandable because in cases of obesity, hyperinsulinemia and IR are associated with increased ovarian androgen production [52]. According to other research, testosterone may exacerbate insulin resistance [53]).
Since vitamin D may influence how glucose is metabolized by improving insulin production and synthesis, increasing the expression of the insulin receptor, and decreasing pro-inflammatory cytokines that could potentially lead to the development of insulin resistance [54].
Vitamin D treatment improved insulin sensitivity in PCOS patients, which may be because it triggers the transcription of the human insulin receptor gene. The promoter of this gene exhibited a vitamin D responsive region, and insulin secretion from beta cells is a calcium-dependent process [55].
However, Amiri et al [56] demonstrated that there was no significant difference between the lean and lean PCOS groups in blood insulin, glucose levels, or HOMA- IR. This could be explained by the fact that certain people meet the requirements for PCOS diagnosis but do not exhibit the typical insulin resistance associated with the disorder. This presentation is known as type II PCOS, or non-insulin resistant PCOS [57].
The results of this study demonstrated statistically significant increases in serum levels of cholesterol, TG, LDL, and VLDL in all obese groups when compared to all lean groups, as well as significant increases in the obese PCO group and lean PCO group when compared to obese control and lean control, respectively. Also, lean PCO and obese PCO had lower HDL levels than lean control and obese control, respectively. In addition, Both the Lean PCO and obese PCO groups supplemented with vitamin D had significantly improvement of their lipid profile in contrast to other PCO groups.
Additionally, we noticed that in all tested groups, serum copeptin had a substantial negative correlation with HDL and a significant positive correlation with cholesterol, TG, LDL, and VLDL.
These results corroborated those of Macut et al [58]. He stated that raised triglycerides and obesity are two aspects of the metabolic syndrome that are linked to greater serum Copeptin levels, along with systolic and diastolic blood pressure.
Additionally, Behmanesh et al [59] showed that rats with PCOS had significantly greater serum levels of triglycerides, cholesterol, and LDL than did controls, but significantly lower levels of high-density lipoprotein (HDL). While triglyceride, cholesterol, and LDL concentrations were

significantly lower in vitamin D-treated rats than in non-treated rats with PCOS, HDL concentrations were significantly greater.

Comparing the lean PCOS group to the lean control group, another study found that there was no significant difference in serum cholesterol, triglycerides, LDL, and HDL values [60]

This could be attributed to the greater incidence of hormonal imbalance and lipid metabolism disease among PCOS participants, to a varied sample size, or to different inclusion criteria [61]

In this study, vitamin D levels were statistically lower in all obese groups than in all lean groups, and lean and obese PCO levels were lower than lean control and obese control, respectively. Additionally, we discovered that serum copeptin levels and vitamin D levels were negatively correlated in all study groups.

According to Wehr et al [62] and our study, metabolic syndrome may be brought on by a vitamin D deficit, and women with PCOS frequently exhibit insulin resistance.

Additionally, Selimoglu et al [63] discovered that most women with PCOS have low vitamin D levels, and vitamin D replacement therapy may help obese women with PCOS who have IR. Additionally, vitamin D insufficiency has a significant impact on reproductive biology, while the aetiology is unknown [64].

Additionally, vitamin D, a fat-soluble vitamin, may be stored and sequestered in adipose tissue because it cannot enter the bloodstream to produce 25(OH)D in the liver. This can result in patients with an excess buildup of adipose tissue having lower plasma levels of 25(OH)D [65].Therefore, vitamin D supplementation may be a part of the comprehensive care for PCOS women [66]. Our study's findings, which showed a link between low vitamin D levels and obesity, insulin resistance, and MS in PCOS women, corroborate this idea.

Nevertheless, according to Mu et al [67], blood vitamin D levels were inversely connected with BMI, body fat, insulin resistance, and hyperinsulinemia. They were also found to be negatively correlated with metabolic problems in PCOS patients.

He cited the absence of a control group in his study as the reason for this. As a result, they are unable to tie low Vitamin D levels to either the metabolic profile of PCOS women specifically or to that of obese women in general.

This study found a significant increase in serum LH in the lean PCO group when compared to the lean control group and to the lean PCO + Vitamin D group, as well as an increase in the obese PCO group when compared to the obese control group and to the obese PCO + Vitamin D group. There was no significant difference in serum FSH between any of the groups, and there was a significant positive correlation between serum copeptin and LH.

This was consistent with research by Behmanesh et al [68], which showed that rats with PCOS had much higher blood LH concentrations than controls while having significantly lower serum FSH concentrations. Vitamin D treatment dramatically decreased the level of LH while considerably increasing the level of FSH in PCOS-affected rats. When compared to the lean control group, Oduwole et al [69] found that the lean PCOS group had significantly higher levels

of LH, but no discernible change in serum FSH. Additionally, the levels of LH in the obese PCOS group were significantly higher than those in the obese control group.

The disruption of the normal gonadotrophin axis is likely caused by increased pituitary sensitivity to gonadotropin releasing hormone (GnRH) secretion patterns rather than GnRH secretion as the reason of LH hypersecretion and FSH reduction or the same level in PCO [70]

This study found that serum testosterone levels significantly increased in the lean PCO group when compared to the lean control group and the lean PCO + Vitamin D groups, as well as in the obese PCO group when compared to the obese control and obese PCO + Vitamin D groups. This is in line with the findings of Behmanesh et al [71] who showed that testosterone concentrations considerably rose a few days after the study's conclusion in rats with PCOS. In rats with PCOS, vitamin D treatment dramatically reduced the testosterone level.

Furthermore, we discovered a strong positive correlation between higher copeptin levels and testosterone levels in PCOS rats. This shows that one of the key factors influencing the rise of copeptin may be the typical hyperandrogenaemia of PCOS. As a result, the hypothesised causes of the elevated copeptin level may not fully be attributable to insulin resistance but may also be attributable to the stimulatory effects of androgen.

Because Letrozole, a non-steroidal aromatase inhibitor, prevents the conversion of androgen substrates to estrogens, high testosterone levels are a reflection of androgen buildup. The pituitary gland's negative feedback on LH production is weakened by the loss in estrogen, which leads to higher levels of LH [72], which further stimulates theca cells to release testosterone.

Common symptoms of PCOS include hyperandrogenism and vitamin D insufficiency, which suggests an inverse relationship between vitamin D concentrations and testosterone levels and that hypovitaminosis D causes hyperandrogenism [73]

In the current study, there was a significant negative correlation between serum copeptin, progesterone, and estrogen as well as a decrease in serum levels of both progesterone and estrogen in the lean PCO group when compared to the lean control group and estrogen in the obese PCO group when compared to the obese control group. Furthermore, we discovered that both the Lean PCO and obese PCO groups supplemented with vitamin D had significantly higher estrogen concentrations than other PCO groups; this may be because the corpus luteum is formed in PCOS-affected rats, which improves aromatase activity and progesterone concentrations. [74]

In accordance with our work, Toosy et al [75] found that the serum progesterone levels in the slim PCOS group were significantly lower than those in the control group. Furthermore, compared to the obese control group, the obese PCOS group had significantly lower serum progesterone levels.

Because the body produces progesterone and estrogen during the ovulation process, anovulation or another major problem with the reproductive organs, absence of ovulation, and a prolonged luteal phase due to hormonal imbalance, such as in PCOS, are the most obvious causes of low progesterone [76]

Vitamin D plays a role in enhancing some important steroidogenic enzymes, and during the normal menstrual cycle, luteinized human granulose cells typically form the corpus luteum, which produces significant amounts of progesterone (and some estrogens) and triggers endometrial changes like decidualization to support a pregnancy. Vitamin D3 potentiates granulosa cell luteinization as indicated by an increase in luteinized granulos [77]. Vitamin D plays a key role in the manufacturing of estrogen in females, and 1,25(OH)2D3 may stimulate the production of progesterone and estradiol [78].

Conclusion:

Copeptin is linked to several metabolic and hormonal alterations associated with polycystic ovarian syndrome and may play a role in the development of this condition and its related comorbidities. Furthermore, vitamin D is inversely correlated with copeptin and its deficiency is linked to PCO, suggesting that vitamin D may be an appropriate supplement to prevent the associated cardiometabolic diseases in PCOS in both lean and obese individuals.

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